Impaired Glucose Metabolism in Primary Aldosteronism is Associated With Cortisol Co-Secretion

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Abstract: CONTEXT Primary aldosteronism (PA) is associated with higher cardiovascular morbidity and metabolic risks. Recent studies report glucocorticoid co-secretion as a relevant phenotype of PA, which could contribute to associated risks, including type 2 diabetes mellitus (T2DM). The relationship between autonomous cortisol secretion (ACS) and glucose metabolism in PA has not been investigated. OBJECTIVE To evaluate the prevalence of impaired glucose homeostasis in PA patients according to cortisol co-secretion. METHODS We performed oral-glucose-tolerance-tests (OGTT) and complete testing for hypercortisolism (1mg-dexamethasone-suppression-test (DST), late-night-salivary-cortisol (LNC), 24hour-urinary-free-cortisol (UFC)) in 161 newly diagnosed PA patients of the German Conn Registry. 76 of 161 patients were reevaluated at follow-up. We compared our results to a population-based sample from the KORA-F4 study matched to the PA participants (3:1) by sex, age, and BMI. RESULTS At the time of diagnosis, 125 patients (77.6%) had a pathological response in at least one of the Cushing screening tests; T2DM was diagnosed in 6.4% of these 125 cases. Patients with pathological DST exhibited significantly higher 2h plasma glucose in OGTT and were significantly more often diagnosed with T2DM than patients with normal DST (20% vs. 0.8%, $p<0.0001$) and matched controls from the KORA study (20.6% vs. 5.9%.; $p=0.022$). PA patients without ACS tended to have higher homeostatic-model-assessment-of-insulin-resistance (HOMA-IR) than KORA control subjects ($p=0.05$). CONCLUSION ACS appears frequently in PA patients and is associated with impaired glucose metabolism, which could increase the risk of T2DM. PA itself seems to enhance insulin resistance.

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cortisol co-secretion in PA and diabetes

IMPAIRED GLUCOSE METABOLISM IN PRIMARY ALDOSTERONISM IS ASSOCIATED WITH CORTISOL CO-SECRETION

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Context: Primary aldosteronism (PA) is associated with higher cardiovascular morbidity and metabolic risks. Recent studies report glucocorticoid co-secretion as a relevant phenotype of PA, which could contribute to associated risks, including type 2 diabetes mellitus (T2DM). The relationship between autonomous cortisol secretion (ACS) and glucose metabolism in PA has not been investigated.
Objective: To evaluate the prevalence of impaired glucose homeostasis in PA patients according to cortisol co-secretion.
Methods: We performed oral-glucose-tolerance-tests (OGTT) and complete testing for hypercortisolism (1mg-dexamethasone-suppression-test (DST), late-night-salivary-cortisol (LNC), 24hour-urinary-free-cortisol (UFC)) in 161 newly diagnosed PA patients of the German Conn Registry. 76 of 161 patients were reevaluated at follow-up. We compared our results to a population-based sample from the KORA-F4 study matched to the PA participants (3:1) by sex, age, and BMI.
Results: At the time of diagnosis, 125 patients (77.6%) had a pathological response in at least one of the Cushing screening tests; T2DM was diagnosed in 6.4% of these 125 cases. Patients with pathological DST exhibited significantly higher 2h plasma glucose in OGTT and were

significantly more often diagnosed with T2DM than patients with normal DST (20% vs. 0.8%, p<0.0001) and matched controls from the KORA study (20.6% vs. 5.9%.; p=0.022). PA patients without ACS tended to have higher homeostatic-model-assessment-of-insulin-resistance (HOMA-IR) than KORA control subjects (p=0.05).
Conclusion: ACS appears frequently in PA patients and is associated with impaired glucose metabolism, which could increase the risk of T2DM. PA itself seems to enhance insulin resistance.

Analysis of 161 patients with primary hyperaldosteronism (PA) and comparison to matched controls revealed that autonomous cortisol secretion in PA is associated with impaired glucose homeostasis.

Introduction

Primary aldosteronism (PA) is the most common endocrine form of secondary hypertension that affects 4.3 to 9.0% of hypertensive patients [1]. Patients with aldosterone excess are at a higher risk of cardiovascular events and metabolic comorbidities in comparison to patients with essential hypertension [2-4]. Recent studies have proven a broader metabolic influence of PA than previously suggested including impaired insulin-secretion [5], insulin-sensitivity [6] and other effects of aldosterone on glucose metabolism [7]. These mechanisms lead to a higher prevalence of the metabolic syndrome and type 2 diabetes mellitus (T2DM) in PA patients [8]. However, some aspects of impairment of glucose homeostasis in PA are still unresolved.

In the past, cortisol co-secretion in primary aldosteronism has been discussed on the basis of several case studies or case series [9-15]. Recently, we have investigated a large multi-center cohort of PA patients and revealed that glucocorticoid co-secretion is a phenotype frequently found in PA which might contribute to associated metabolic risks [16], including a higher risk of cardiovascular events as shown by Nakajima et al. [17]. Specifically, our findings suggest that cortisol excess in PA also plays a role in impaired glucose metabolism. However, further investigations of the underlying mechanisms have not been undertaken yet.

Therefore, we analyzed autonomous cortisol secretion (ACS) and glucose metabolism in detail in newly diagnosed PA patients of the German Conn Registry. In addition, we used the population-based Cooperative Health Research in the-region of Augsburg (KORA)-F4 study with a 3:1 matching by sex, age, and BMI for comparison.

Methods

Study Population

The German Conn Registry

The study population consists of 161 patients that were recruited in two centers (Munich; Berlin) of the German Conn Registry. The German Conn Registry is a multicenter-registry that investigates therapy, comorbidities and the longtime-outcome in PA-patients throughout Germany since 2008 [18]. The investigated cohort was obtained between February 2013 and April 2017.

For inclusion in the registry, patients had to meet the diagnostic criteria for PA, as stated in the guidelines of the Endocrine Society [19]. Patients were usually screened with high blood pressure and abnormal aldosterone to renin ratio (ARR) and then underwent one or more confirmatory tests (saline infusion, fludrocortisone suppression, captopril test, oral salt loading test with elevated excretion of aldosterone and metabolites in urine). Before implementation of those tests (including standard oral glucose tolerance test (OGTT) and complete testing for hypercortisolism), anti-hypertensive medication was changed whenever possible or indicated (deduction of beta-blockers, central-alpha-agonists, angiotensin-converting-enzyme-blocker, angiotensin-receptor-blocker for at least one week and mineralocorticoid receptor antagonists (MRA) for at least four weeks prior testing), in order

to prevent influences on renin-angiotensin-aldosterone-system and thus test results. The diagnosis was then made decentralized in the synopsis of all clinical and laboratory findings according to the guidelines of the Endocrine Society.

Subtype identification (aldosterone producing adenoma (APA) vs. bilateral adrenal hyperplasia (BAH)) was performed via adrenal imaging (MRI or CT) and adrenal vein sampling (AVS), which was realized in 95.7% (n=154) of patients and successful in 89.6% (n=138) of those. During AVS, blood is obtained from both adrenal veins and a peripheral vein. We assessed blood samples for hormone levels of both aldosterone and cortisol, in order to correct a dilution effect and confirm correct catheterization [19]. Catheterization was performed without cosyntropin stimulation and was considered successful when cortisol levels in both adrenal veins were at least twice as high as in vena cava inferior. Unilateral aldosterone excess was considered to be present in patients with a lateralization index ((aldosterone left/cortisol left)/(aldosterone right/cortisol right) or vice versa) of at least 4:1.

For the present study, all PA patients underwent a standard OGTT and complete testing for hypercortisolism, including 1mg dexamethasone suppression test (DST), 24hour urinary free cortisol (UFC) and late-night salivary cortisol (LSC) at baseline visit. Patients with missing data for aldosterone, renin, potassium, OGTT or blood pressure were excluded. Also, PA patients with a previously known diabetes mellitus were excluded. We assessed glucose metabolism by laboratory measurement and OGTT, according to the American Diabetes Association [20]. 76 of 161 patients had a follow-up visit one year after therapy initiation with MRA or adrenalectomy (ADX). The Ethics Committees of the Klinikum of the University of Munich and of the Conn´s registry participating centers approved the protocol. Personal data protection laws were strictly adhered to. All included patients gave their written informed consent.

KORA-Study

The KORA-F4 study is the 7-year-follow-up examination of the population-based KORA-S4 study [21, 22]. Baseline-examinations of KORA-S4 were conducted in 1999-2001 (n=4261) in men and women aged 25-74 years. In 1353 participants aged 55-74 years an OGTT was performed at baseline [23]. 3080 participants were re-investigated in 2006-2008 as part of KORA-F4. These participants comprise the basis for the present analyses. Investigations included a standard medical interview, physical examination, blood withdrawal, and an OGTT in all individuals without known T2DM after an overnight fasting period of ≥8h [24, 25].

The KORA studies were approved by the Ethics Committee of the Bavarian Medical Association. Written informed consent was obtained from all participants, and data protection policies strictly adhered to.

Definitions and laboratory measurements

In PA patients as well as in controls standard laboratory measurements were performed immediately and decentralized. Measurements of cortisol and ACTH were performed as previously described [26]. Measurement of serum cortisol and plasma ACTH was performed with Solid Phase Antigen linked Technique (Cortisol, SPALT, Liaison, DiaSorin, Saluggia, Italy) and chemiluminescence immuno-assay (ACTH, CLIA, Liaison, DiaSorin). Within- and between-assay coefficients of variation were below 5 and 7% (Cortisol) and below 13% (ACTH) respectively. Urinary cortisol measurement was performed with chemiluminescence immunoassay (ADVIA Centaur, Siemens) with within- and between-assay coefficients variations below 7%. Salivary cortisol was measured by a luminescence immunoassay (Cortisol LIA, IBL, Hamburg, Germany) with within- and between-assay coefficients variations below 9% and 6%.

In order to test for hypercortisolism, we performed 1mg DST and acquired LSC, as well as 24hour UFC in all patients at baseline visit. Autonomous cortisol co-secretion (ACS) as an

indicator for hypercortisolism was assumed when DST, LSC or UFC were above normal reference values (≥51 nmol/l; >1.45 ng/ml; >150 µg/24hours, respectively). Reference values were determined following the Guidelines of the Endocrine Society [27].

Blood pressure (BP) was measured up to three times on each arm after at least 5 minutes of resting with standard sphygmomanometers. Body mass index (BMI) was calculated as body weight (kg) per heights^2 (m^2).

The homeostasis model assessment of insulin resistance (HOMA-IR) score was calculated (fasting serum insulin (mU/l) * fasting plasma glucose (mg/dl) /405) for patients of the German's Conn Registry and (fasting serum insulin (mU/l * fasting plasma glucose (mmol/l))/22.5) for KORA-F4 participants. HOMA-β, as estimate for β-cell function, was calculated using the following formula: 20 × fasting insulin (µIU/ml) / (fasting plasma glucose (mmol/l) - 3.5). In order to investigate glucose metabolism, we performed an OGTT at baseline in all patients and measured HbA1c (%) in patient's blood samples. Only non-diabetic Conn patients and non-diabetic KORA participants (= no intake of antidiabetic drugs, no diagnosis by another physician, no reported T2DM by the patient) received an OGTT.

OGTT was performed after an overnight fast of at least 8 hours with 75g glucose dissolved in 300ml of water. Blood samples were obtained before glucose load (fasting plasma glucose; FPG) and 120minutes (2h glucose) after glucose load. Patients were diagnosed with either T2DM, prediabetes or normal glucose metabolism, according to the American Diabetes Society [20]: newly detected T2DM was defined if HbA1c ≥6.5%, FPG ≥ 126 mg/dl or glucose at 120 minutes of OGTT ≥ 200mg/dl. Prediabetes was defined by HbA1c (5.7-6.4%) or OGTT result in either impaired FPG (100-125 mg/dl in OGTT) or impaired glucose tolerance (IGT 140-199 mg/dl in OGTT). The term prediabetes includes isolated impaired FPG, isolated IGT and both combined. All KORA participants with clinically diagnosed diabetes who did not receive an OGTT were included in the diabetes group in analyses regarding diabetes prevalence.

Matching and Statistical analysis

Matching was performed sex-stratified and further matching variables were age (+/- 5 years) and BMI-category (<25; 25-29; ≥30kg/m^2). In order to be able to achieve a 3:1 matching, four young patients (<32 years) from the German-Conn-Registry had to be excluded. Blood-pressure was not chosen as a matching variable, as KORA participants are population-based whereas PA patients form a hypertensive cohort. KORA participants with type 1 or type 2 diabetes or drug induced diabetes were excluded, as well as participants with ARR >20 and those with missing values of systolic or diastolic blood pressure, fasting glucose or renin concentrations. This 3:1 matching resulted in 471 matched KORA participants (controls) for 157 PA patients. Differences between PA patients and controls were obtained using conditional regression analysis.

Statistical analysis was carried out using IBM SPSS Statistics 25.0 (IBM, Ehningen, Germany). Data are displayed as mean and standard deviation (mean ± SD) for normally distributed continuous data, and as median; 25th and 75th percentile for continuous variables without normal distribution. Categorical variables are displayed as percentage or numbers. Variables were assessed for normal distribution using Shapiro-Wilk test.

To compare normal and pathological subgroups we used either Mann-Whitney U test or unpaired t-test for continuous data and Chi2 test for categorical variables. For paired data comparing baseline and follow-up visit, we used McNemar for categorical data and Wilcoxon signed rank test or paired t-test for continuous data. T-Tests (paired or unpaired) was only applied if normal distribution in both subgroups was given. Differences were considered statistically significant when $P \leq 0.05$.

Results

In 161 investigated PA patients, ACS was identified in 77.6% (n=125; 61 with one, 58 with two, and 6 with three pathological tests), whereas 22.4% (n=36) showed a normal response in all three tests for hypercortisolism (noACS). We found no differences in age, BMI, BP, potassium or lipid parameters between the groups (**Table 1**). However, women with ACS had a significantly higher WHR than women without ACS (**Table 1**). PA patients with ACS had significantly higher ARR in comparison to the noACS subgroup (79.2; 43.6-141 vs. 60.0; 30.6-94.9; *p=0.029*), and showed a higher lateralization rate (49.6% vs. 30.6%; *p=0.043*). T2DM was diagnosed in 6.4% of the PA patients with ACS, while no T2DM was apparent in any patients of the noACS subgroup (*p=0.119*) (**Figure 1**). The prevalence of prediabetes (27.8% vs 27.2%; p=0.945) and of the metabolic syndrome (19.4% vs. 16.0%; *p=0.626*) was not different between the noACS and the ACS subgroup (**Figure 1**). Also, no differences were detected regarding FPG, 2h plasma glucose levels or HOMA-IR.

In PA patients with ACS we found significant differences in ARR (156±156 vs 112±185; *p=0.006*) and potassium (3.5±0.4 vs 3.7±0.3 mmol/l; *p=0.033*) levels between patients with unilateral and bilateral disease, however no differences were seen in parameters of glucose homeostasis. In PA patients with noACS, potassium (3.3±0.4 vs 3.7±0.3 mmol/l; *p=0.005*) significantly differed between patients with unilateral and bilateral disease, however also no differences were seen in parameters of glucose homeostasis.

We further analyzed ACS depending on the DST results only: 35 of 161 patients (21.7%) were found to have a pathological response in DST (pathDST). PA patients with pathDST displayed a tendency towards a higher 2h plasma glucose levels (*p=0.053*) in OGTT than PA patients with normalDST (**Figure 2**). This resulted in a significantly higher prevalence for T2DM in the pathDST-subgroup (20% vs. 0.8%, *p<0.0001*). However, FPG, HbA1c, HOMA-IR and the prediabetes prevalence were not different between pathDST and normalDST subgroups. Also, no differences were seen in other clinical and lab parameters.

To further explore these findings, we matched 158 patients of our cohort 1:3 with participants of the KORA-F4 study. We further aimed to differentiate between effects of aldosterone excess and ACS on glucose homeostasis by using the KORA study. In a first step, we compared the characteristics of PA patients without ACS (n=35) to matched KORA controls (n=105) (**Table 2)**. PA patients without ACS showed no difference in fasting plasma glucose or 2h plasma glucose levels in OGTT compared to matched controls. However, HOMA-IR was higher with a borderline significance (*p=0.051*) than in matched controls (**Table 2**) suggesting insulin resistance due to hyperaldosteronism. PA patients without ACS showed no difference in HbA1c, but a significantly higher WHR than matched controls (**Table 2**). Furthermore, LDL cholesterol (115±29.9 vs. 135±39.4; *p=0.010*) and total cholesterol levels (194±33.5 vs. 217.5±40.5; *p=0.004*) were significantly lower in PA patients without ACS than controls.

The next step was to compare PA patients with ACS, proven by pathological DST (pathDST), (n=34) with matched controls from the KORA study (n=102) (**Table 3**). PA patients with ACS showed no difference in fasting plasma glucose or HOMA-IR compared to matched controls. However, the 2h plasma glucose levels in OGTT were significantly higher (*p=0.001*) than in matched controls (**Table 3**) indicating impaired glucose tolerance. This subgroup of PA patients with ACS also presented with significantly lower HbA1c and higher WHR (**Table 3**), as well as lower triglycerides (78.5; 57.5-127 vs. 98.5; 64.0-253; *p=0.041*) and cholesterol levels (201±34.6 vs. 218±41.6; *p=0.041*) than matched controls.

We also compared 63 PA patients with a pathLSC to matched KORA individuals (n=189). Thereby, we detected lower fasting plasma glucose levels in PA patients with pathLSC compared to matched controls, while no difference in HOMA-IR was evident. However, similar to patients with pathDST, the 2h plasma glucose levels in OGTT were also

significantly higher (*p=0.002*) in PA patients with pathLSC than in matched controls, whereas they presented with significantly lower HbA1c and higher WHR. The same pattern (significantly (*p=0.0004*) higher 2h plasma glucose levels in OGTT) was found in 93 PA patients with pathUFC compared to their matched KORA controls (n=279).

We further evaluated additive effects when considering multiple pathological tests. Two pathological test results for hypercortisolism showed greater significance regarding differences in 2h plasma glucose in OGTT (*p=0.001*) compared to matched KORA subjects. Due to a small number of patients with three pathological test results for hypercortisolism (n=6), statistical analysis was not performed.

76 of 161 patients received follow-up visit one year after initiation of therapy (32.9% ADX; 63.2% MRA; 3.9% other therapies) and characteristics are showed in **Table 4**. At follow-up we documented post-operative adrenal insufficiency in 5 of the 25 ADX patients with no difference in prevalence between ACS and noACS patients. Those 5 patients temporarily received hydrocortisone treatment; and no adrenal crisis occurred. At follow-up, patients showed a significant (*p<0.001*) decrease in BP, an increase in potassium and decrease in ARR. BMI and WHR did not change; however, HbA1c levels were significantly higher at follow-up. 23.7% of the follow-up patients were in the noACS-subgroup (n=18), and 76.3% in the ACS-subgroup (n=58).

In PA patients without ACS no significant changes in prevalence of prediabetes or T2DM were seen between baseline and follow-up (**Figure 3a**). Also, in PA patients with ACS, no significant changes in prevalence of prediabetes or T2DM were observed (**Figure 3b**), even when some patients improved from the T2DM to the prediabetes and from the prediabetes group to the normal-glucose-homeostasis group, other patients worsened on follow-up to the prediabetes or T2DM group.

Discussion

Patients with PA are characterized by a significantly increased risk to develop further comorbidities, including cardiovascular, renal and cerebrovascular disease. These risks are usually significantly higher than in hypertensive patients and thus are attributed to aldosterone excess [2-4].

Among others, APA induced hypokalemia is stated as a secondary cause for T2DM by the American Diabetes Association [20]. Diabetes prevalence in PA ranges from 8.2% to 23%, depending on the study population and the applied diagnostic criteria [8, 28-31]. Even though some studies could not show an increased risk of T2DM in PA patients [29, 31] other studies are in favor of this assumption. For example, a retrospective cohort of the German Conn's registry established a significantly increased risk for T2DM (23% vs. 13%, *P*=0.03) in comparison to hypertensive control subjects [30]. These results were confirmed by a prospective study of Hanslik et al. who could demonstrate a prevalence of 17.2% for T2DM in PA patients, which was significantly higher than in their population-based control cohort [8].

Different mechanisms that lead to glucose impairment in PA have been discussed [32]. One major contributing factor for PA patients to develop T2DM seems to be hypokalemia, which impairs insulin release by pancreatic beta-cells [33]. Other mechanism that lead to impaired glucose tolerance in PA include reduced insulin sensitivity and impaired insulin signaling and thus reduced glucose uptake in peripheral tissue, including liver, skeletal muscle and adipose tissue [34-36]. Furthermore, aldosterone induces reactive oxygen species (ROS) and increases insulin-like growth-factor-1 expression, ultimately causing endothelial dysfunction which leads to an impaired glucose diffusion [37, 38]. Finally, adipose tissue expresses both mineralocorticoid receptor (MR) and glucocorticoid receptor (GR). Due to absence of 11β-hydroxysteroid dehydrogenase (11β-HSD) type 2 in adipocytes, the MR is thought to be at least partly occupied by cortisol originating from blood or from intracellular activation of

cortisone by 11β-HSD type 1 [39]. Therefore, we hypothesize that cortisol cosecretion in PA might alter adipocyte differentiation and subsequently influence insulin sensitivity and glucose homeostasis.

One further aspect of PA that might result in disturbances of glucose metabolism is glucocorticoid co-secretion. First, a possible cortisol co-secretion in PA patients could only be shown in a few patients of small sized retrospective cohorts [10, 17]. However, recently larger studies suggest that glucocorticoid excess (autonomous cortisol secretion, ACS) is a frequent finding in PA [16, 40]. We demonstrated a correlation of 24-hour glucocorticoid output with markers of insulin resistance - including fasting insulin, insulin after OGTT and HOMA-IR. Thus, indicating that glucocorticoid co-secretion might affect glucose homeostasis.

However, we now present the first study to evaluate the impact of ACS on the prevalence of T2DM in PA in comparison to a 1:3 matched control group. We prospectively investigated ACS with the help of three different tests for hypercortisolism and set them into context with results of OGTT. We could identify 125 patients (77.6%) with ACS in at least one of the tests and 34 patients (21.1%) with pathological response in DST alone. Until now smaller studies estimated the prevalence of cortisol excess in PA patients at 3.9%-33.3%, depending on the diagnostic criteria used [9-11, 17]. Most of the studies used DST as their main diagnostic criteria in combination with another feature of ACS, formerly named subclinical hypercortisolism. On this basis, we prove that ACS is a frequent finding in PA and should be considered as another factor of comorbidities in PA patients. It is important to point out that mild autonomous cortisol secretion (MACE) contributes to lower bone mineral density, and might have the same effect in our PA patients with ACS[41].

In our cohort, we could show that PA patients with ACS have a higher prevalence of T2DM than sex, age, and BMI-matched controls. In contrast to this, PA patients without ASC – and thus with aldosterone excess alone - could not be diagnosed with T2DM, but showed higher HOMA-IR values than their matched KORA-controls. HOMA-IR is known as a marker of hepatic glucose and insulin in the fasting state [42]. This leads to the assumption that aldosterone might directly affect hepatic insulin-resistance. Previous studies have shown that aldosterone administration increases FPG and leads to an increased expression of gluconeogenetic enzymes [43], which might lead to the observed deterioration of HOMA-IR values.

Investigating the effect of ACS in PA on glucose homeostasis, we detected that PA patients with proven ACS showed higher 2h glucose levels during the OGTT and a higher prevalence of T2DM compared to matched KORA-controls. Thus, it seems that an additional ACS impairs glucose tolerance in the peripheral tissues. Possible mechanisms might include impairment of insulin-dependent glucose uptake in peripheral tissue [44] or enhanced gluconeogenesis via different mechanisms, including further induction of gluconeogenetic enzymes [45]. Furthermore, the presence of more than one abnormal test depicting cortisol excess seems to be associated with a greater risk of impaired glucose tolerance.

Interestingly, parameters of glycemia of our patients did not improve at follow-up after 12 months. This is in contrast to the study by Catena et al.[46] which showed improved glucose homeostasis after 6 months of treatment. However, this study included only 47 PA patients, and glucose homeostasis worsened again over a longer follow-up [46]. In our patients treated with ADX the source of aldosterone and glucocorticoid excess seems to be removed and thus usually these patients perform better in follow-up studies than patients treated with MRA. Still, some patients treated with ADX do not show complete biochemical cure. The reasons for this were summarized recently and comprise surgery based on CT subtyping, different accuracy of simultaneous and sequential AVS or usage of different selectivity and lateralization indexes [47]. In our study we suspect other possible influences such as increased

age, unhealthy diet, physical inactivity or stress, or the small number of patients with follow-up data.

One limitation of the present study is that only 12 patients without T2DM at baseline agreed to redo an OGTT and ACS was not reevaluated at follow-up so that effects of glucocorticoids on glucose metabolism over time might be distorted. In addition, LSC, DST and UFC were not repeated after treatment, and, thus, we cannot rule out that mild cortisol excess persisted in some patients. Furthermore, we did not investigate other factors that might influence the development of glucose intolerance, such as family history of diabetes or drug-induced diabetes. The strengths of our study are that the German Conn Registry, as well as the KORA-F4 study, collects data in a prospective and standardized manner. We can present a large-sized and well-characterized cohort with follow-up investigations. We could match our PA patients in a 1:3 sex, age, and BMI-based matching to participants from a population-based study, in order to achieve a case-control-design. A further limitation of our study might be that in PA patients with ACS the cortisol hypersecretion might interfere with the interpretation of the AVS data because up to now cortisol, and not plasma metanephrine[48], is used as normalization factor in AVS aldosterone measurements.

In conclusion, we show that our PA cohort possesses a high proportion of patients with ACS. We describe that T2DM and impaired 2h plasma glucose in OGTT is more prevalent in PA patients with ACS than in controls matched for sex, age, and BMI. These results give further evidence for the "Connshing" syndrome and point out the relevance for further investigation of the underlying mechanisms and especially associated risks such as T2DM.

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Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Figure 1: Frequencies in glucose metabolism alterations of patients with primary aldosteronism at baseline visit and complete testing for hypercortisolism (n=161). No autonomous cortisol secretion (ACS) – patients with normal test results regarding hypercortisolism; ACS – patients with pathological response in at least one test for hypercortisolism (dexamethasone suppression test, late-night salivary cortisol and/or urinary free cortisol).

Figure 2: Plasma glucose levels in oral glucose tolerance test (OGTT) of patients with primary aldosteronism (PA) depending on cortisol levels after dexamethasone suppression test (DST), (autonomous cortisol secretion: cortisol levels >50nmol/l). Lines at 126mg/dl and 200mg/dl indicating cut-offs for diagnosing diabetes mellitus by fasting plasma glucose and glucose after 120 minutes. Mild outliers (1.5-3xIQR) are displayed as circles and extreme outliers (> 3xIQR) displayed as stars.

Figure 3: Frequencies of glucose metabolism alterations in patients with primary aldosteronism (PA) **a)** without (n=18) and **b)** with (n=58) autonomous cortisol secretion (ACS) in at least one test at baseline and at follow up-visit after one year. Dashed lines indicate changes within different subgroups, based on diagnosis at baseline visit.

Table 1: Characteristics of primary aldosteronism (PA) patients with autonomous cortisol secretion (ACS) in at least one test and of PA patients without ACS (patients with normal test results regarding hypercortisolism). WHR = waist-to-hip ratio (analyzed separately for men and women due to different optimal values in males and females); BMI = body mass index; BP= blood pressure; DST = dexamethasone suppression test; a= two missing values; b= three missing values; c= six missing values; d= ten missing values; e=12 missing values; f=30 missing values. Data are displayed as mean ± SD for normally distributed continuous data, and as median; 25th and 75th percentile for continuous variables without normal distribution.

Characteristics	No ACS	ACS	*P*
n (%)	36 (22.4)	125 (77.6)	**0.000**
Male n (%)	12 (33.3)	71 (56.8)	**0.013**

Age (years)	49.3±11.3		51.9±11.1		0.218	
BMI (kg/m^2)	27.0±4.7		27.3±5.1 (26.5; 23.5-29.0)		0.914	
Systolic BP (mmHg)	143±18.0		149±17.6 (147; 136-158)		0.092	
Diastolic BP (mmHg)	93.0±11.6		92.4±10.3		0.794	
Potassium (mmol/l)	3.6±0.4 (3.7; 3.4-3.9)		3.6±0.4		0.979	
WHR	female (n=24)	male (n=12)	female (n=54)	male (n=71)	female	male
	0.8±0.1[a]	0.9±0.1[b]	0.9±0.3[c] (0.9; 0.8-1.0)	1.0±0.1[d]	**0.036**	0.670
HbA1c (%)	5.2±0.4 (5.2; 4.9-5.5)		5.2±0.4 (5.2; 4.9-5.4)		0.919	
HDL-cholesterol (mg/dl)	63.4±17.1		60.0±15.8 (59.0; 46.0-71.0)		0.329	
LDL-cholesterol (mg/dl)	115±29.8		119±34.1		0.533	
Triglycerides (mg/dl)	98.8±44.8 (91.0; 61.3-137)		95.0±45.3 (88.0; 62.5-115)		0.633	
Total cholesterol (mg/dl)	194±33.0		194±34.9		0.983	
Statin therapy n (%)	4 (11.1)		11 (8.8)		0.674	
basal cortisol (µg/dl)	11.1±4.9 (10.2; 7.7-12.9)		13.5±5.3[a] (13.0; 10.0-16.5)		**0.004**	
basal ACTH (pg/ml)	19.0±20.0[e] (13.0; 7.0-23.5)		19.0±17.5[f] (15.0; 8.5-23.0)		0.776	
Cortisol after 1mg DST(nmol/l)	33.1±9.0		55.0±49.2 (41.4; 30.3-57.9)		**0.001**	
late night salivary cortisol (ng/ml)	0.8±0.3		1.8±1.3 (1.5; 0.9-2.3)		**0.000**	
Urinary free cortisol (µg/24h)	85.9±37.3		208±342 (171; 103-258)		**0.000**	
Hypo-/Normokalaemic PA (%)	61.1/38.9		64.0/36.0		0.751	

Table 2: Characteristics of primary aldosteronism (PA) patients without autonomous cortisol secretion (ACS) and matched controls from the KORA cohort. WHR = waist-to-hip ratio; BMI = body mass index; BP= blood pressure; OGTT = oral glucose tolerance test; m = matching variables; a= 4 missing values; b= 2 missing values. Data are displayed as mean ± SD for normally distributed continuous data, and as median; 25th and 75th percentile for continuous variables without normal distribution.

Characteristics	**PA without ACS (n=35)**	**KORA (n=105)**	***P***
male n (%)	12 (34.3)	36 (34.3)	*m*
Age (years)	50.0±10.7	50.2±10.8 (49.0; 43.0-57.0)	*m*
BMI (kg/m^2)	27.0±4.8	26.7±5.5 (26.2; 21.8-29.6)	*m*
Systolic BP (mmHg)	143±18.2	118±18.3	**0.000**
Diastolic BP (mmHg)	92.9±11.7	75.5±10.2	**0.000**
WHR	0.9±0.1[a]	0.8±0.1 (0.8; 0.8-0.9)	**0.011**
Potassium (mmol/l)	3.6±0.4 (3.7; 3.4-3.9)	4.2±0.3	**0.000**
HbA1c (%)	5.2±0.4 (5.2; 4.9-5.5)	5.4±0.8 (5.3; 5.1-5.5)	0.054
Fasting plasma glucose (mg/dl)	89.1±10.5 (87.0; 80.0-95.0)	94.9±27.5 (90.0; 85.5-97.0)	0.122
2h OGTT glucose (mg/dl)	109±31.0	110±41.0 (100; 84.0-126)[b]	0.885
HOMA-IR	2.0±2.1 (1.2;0.9-2.3)	1.2±1.6 (0.8; 0.5-1.3)	0.051
HOMA-β (%)	137±185 (92.5; 57.3- 133)	118±55.6 (106; 73.7-150)	0.378
Diabetes mellitus n (%)	0 (0)	5 (4.8)	0.432
Prediabetes n (%)	10 (28.6)	24 (22.9)	0.532

Table 3: Characteristics of primary aldosteronism (PA) patients with pathological response in dexamethasone suppression test (pathDST) and matched controls from the KORA cohort. WHR = waist-to-hip ratio; BMI = body mass index; BP= blood pressure; OGTT = oral glucose tolerance test; m = matching variables; a= 2 missing values; b= 4 missing values; Data are displayed as mean ± SD for normally distributed continuous data, and as median; 25th and 75th percentile for continuous variables without normal distribution.

Characteristics	**PA with pathDST (n=34)**	**KORA (n=102)**	***P***
male n (%)	17 (50.0)	51 (50.0)	*m*
Age (years)	54.9±10.6	55.0±10.8 (57.0; 46.0-64.3)	*m*
BMI (kg/m^2)	26.0±4.6 (25.2; 23.0-27.7)	26.0±4.5 (35.0; 22.7-28.5)	*m*
Systolic BP (mmHg)	151±21.1 (151; 140.5-161)	123±15-5	**0.000**
Diastolic BP (mmHg)	94.1±12.4	75.6±9.2	**0.000**
WHR	1.0±0.4 (0.9; 0.8-1.0)[a]	0.9±0.1	**0.000**
Potassium (mmol/l)	3.5±0.4	4.2±0.3	**0.000**
HbA1c (%)	5.3±0.5	5.5±0.4 (5.5; 5.2- 5.6)	**0.013**
Fasting plasma glucose (mg/dl)	95.0±16.5 (92.0; 86.8-99.0)	96.7±16.6 (93.0; 87.0-103)	0.579
2h OGTT glucose (mg/dl)	139±51.0 (127; 101-177)	107±33.3 (99-5; 86.0-120)[b]	**0.001**
HOMA-IR	1.7±1.2 (1.5; 0.8-2.2)	1.6±2.3 (0.9; 0.5-1.4)	0.725

HOMA-β (%)	88.5±56.2 (71.4; 44.8-122)	108±53.8 (102.3; 72.2-130)[b]	0.056
Diabetes mellitus n (%)	7 (20.6)	6 (5.9)	**0.022**
Prediabetes n (%)	8 (23.5)	42 (41.2)	0.060

Table 4: Characteristics of 76 patients with primary aldosteronism (PA) patients at baseline and at one-year follow-up. Number of hypertensives at follow-up does not include mineralocorticoid receptor antagonists (MRA). ADX = adrenalectomy; (no)ACS = no autonomous cortisol co-secretion in at least one test for hypercortisolism; BP = blood pressure; PAC = plasma aldosterone concentration; PRC = plasma renin concentration; ARR = aldosterone renin ratio; WHR = waist-to-hip ratio; BMI = body mass index; BP= blood pressure; a= one missing value; b= 5 missing values; c= 2 missing values; d= 4 missing values; Data are displayed as mean ± SD for normally distributed continuous data, and as median; 25th and 75th percentile for continuous variables without normal distribution.

Variables	**ADX (n=25)**			**MRA (n=48)**			**Others (n=3)**	
	Baseline	**Follow-up**	***P***	**Baseline**	**Follow-up**	***P***	**Baseline**	**Follow-up**
noACS/ACS n (%)	3/22 (12/88)		-	15/33 (31.2/68.8)		-	2/1 (66.7/33.3)	
(unilateral/bilateral/unknown)	(25/0/0)			(9/35/4)			(2/0/1)	
male n (%)	11 (44.0)		-	25 (52.1)		-	2 (66.7)	
Systolic BP (mmHg)	145±13.4	137±17.0 (136; 123-149)	0.071	148±21.0 (145; 133-159)	131±16.7 (128; 120-138)	**0.000**	148±21.0	131±12.1
Diastolic BP (mmHg)	89.8±8.0	89.2±9.4	0.799	92.1±11.1	87.4±9.5	**0.001**	100±12.0	90.3±6.6
Potassium (mmol/l)	3.4±0.3	4.2±0.4	**0.000**	3.6±0.3	4.0±0.4	**0.000**	3.9±0.4	4.2±0.1
PAC (ng/l)	441±505 (234; 137-466)	69.7±32.3	**0.000**	180±112 (145; 110-195)	255±161 (225; 155-301)	**0.003**	210±124	258±43.7
PRC (ng/l)	3.1±2.3 (2.2; 1.2-4.6)	15.6±25.7 (7.6; 3.8-16.3)	**0.000**	3.9±4.3 (2.5; 1.4-4.2)	10.5±14.1 (5.8; 2.3-14.5)	**0.000**	2.9±2.2	14.1±22.3
ARR	221±296 (112; 43.4-289)	14.2±15.8 (7.8; 3.9-19.8)	**0.000**	82.4±90.1 (58.8; 31.6-97.7)	55.8±53.8 (30.7; 20.8-65.7)	0.076	103±79.8	136±115
Number of hypertensives	1.7±0.8 (2.0; 1.5-2.0)	1.4±1.2 (1.0; 0.0-2.0)	0.160	1.6±0.8 (2.0; 1.0-2.0)	1.5±1.1 (1.0; 1.0-2.0)	0.471	1.3±0.6	1.7±1.2
BMI	28.9±6.3 (28.6; 23.9-30.8)	29.0±6.3 (28.6; 24.2-30.9)	0.455	26.8±5.0 (25.3; 23.4-28.7)	26.9±5.3 (257; 23.2-29.2)	0.919	26.7±5.8	26.4±5.2
WHR	1.0±0.2 (1.0; 0.9-1.0)[a]	1.0±0.1[b]	0.126	0.9±0.1[c]	0.9±0.1[d]	0.875	1.5±1.0	0.8±0.1[a]
HbA1c (%)	5.4±0.4	5.6±0.4	**0.002**	5.2±0.4	5.4±0.4[a]	**0.006**	5.0±0.4	5.2±0.3
HDL-cholesterol (mg/dl)	59.8±13.5	54.0±13.1[a]	**0.008**	62.3±19.1	58.7±18.2 (58.5; 46.3-69.8)	**0.031**	60.7±11.9	58.0±20.5
LDL-cholesterol (mg/dl)	121±41.0	127±35.5[a]	0.490	117±32.7	115±37.8	0.576	104±4.2	114±12.5
Triglycerides (mg/dl)	90.2±44.3 (80.0; 56.5-110)	114±45.0	**0.001**	101±43.8	132±87.2 (120; 73.3-172)	**0.000**	88.0±9.1	130±102
Total cholesterol (mg/dl)	195±40.2	200±35.2	0.585	195±32.8	194±39.6 (183; 163-219)	0.400	179±9.1	194±26.5

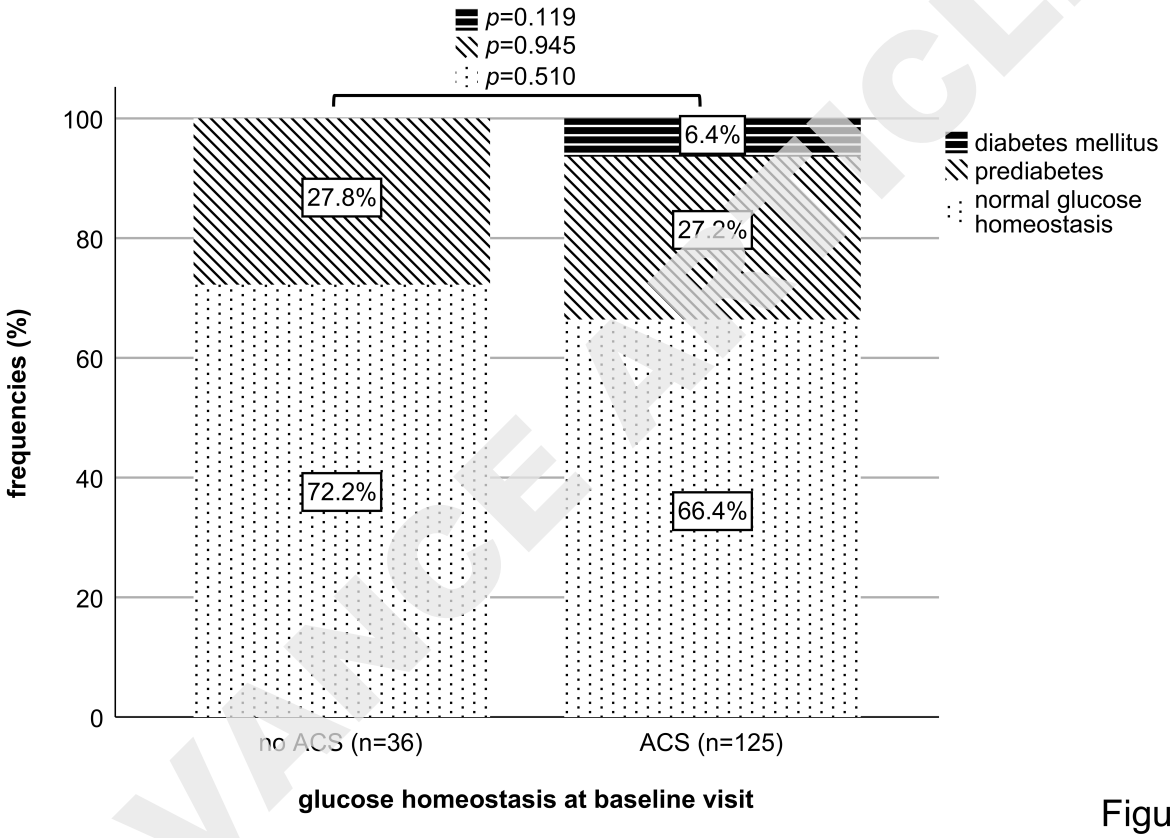

Figure 1

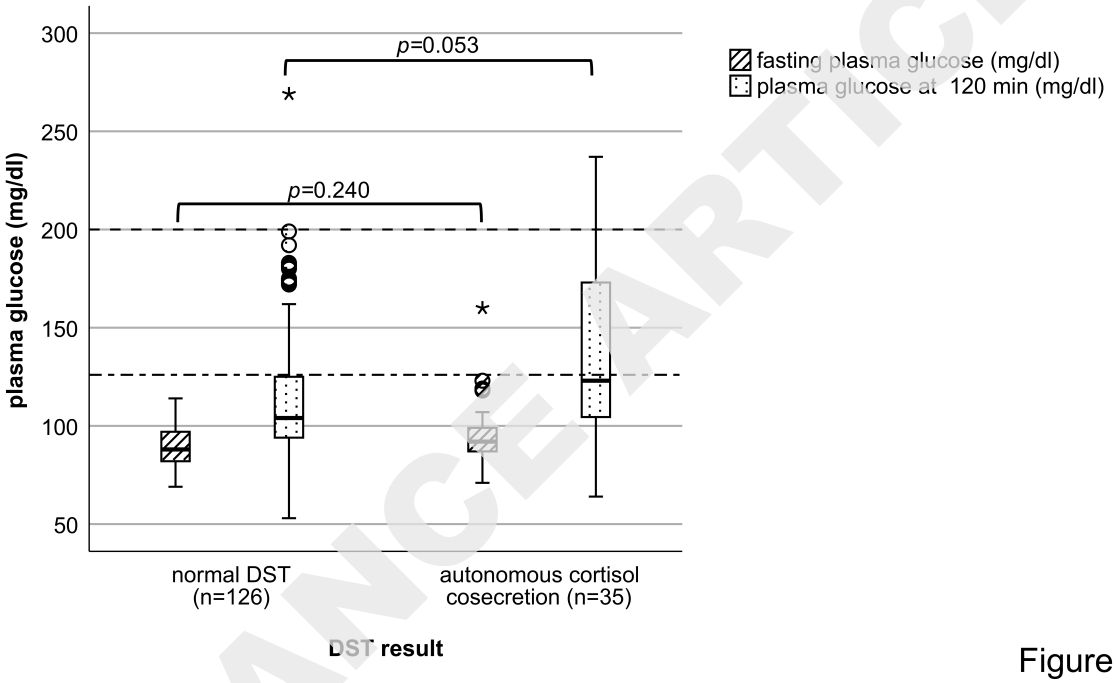

Figure 2

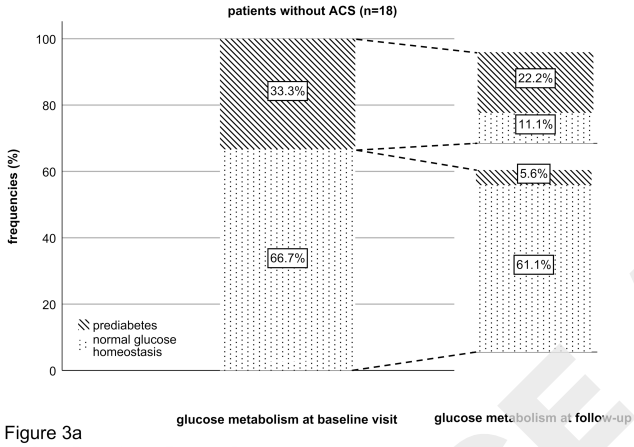

Figure 3a

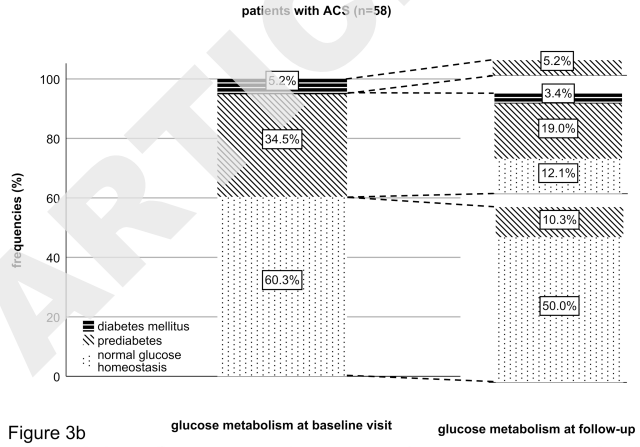

Figure 3b